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Review

The role of prolactin and growth hormone in mammary gland development

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Abstract

Development and differentiation of the mammary gland occur primarily during pregnancy. Females homozygous (−/−) for the null mutation of the PRL receptor (PRLR) gene are sterile due to a complete failure of blastocysts to implant. In progesterone-treated mice pregnancy is rescued but the mammary gland is severely underdeveloped. Interestingly, females hemizygous for the PRLR (+/−) in their first lactation show an almost complete failure to lactate. This phenotype disappears in the second and subsequent pregnancies in inbred 129/Sv mice but is maintained in inbred C57BL/6 mice. In GH receptor (GHR) KO mice litter size is markedly decreased, probably due to an ovarian defect. To assess the relevance of the GH and PRLRs in the mammary gland development, GHR and PRLR null epithelia were transplanted into cleared fat pads of wild-type mice. Such studies show that epithelial GHR is not required for functional mammary development. In contrast, epithelial PRLRs are required for mammary development and milk protein gene expression during pregnancy. Since ductal development is impaired in GHR −/− mice, it appears that GH signals through the stromal compartment. In summary, it is now established that GH and PRL activate Stat5 in separate compartments, reflecting their specific roles in ductal and alveolar development and differentiation.

Keywords: Epithelial cells; Stroma; Receptor knockout; Transplantation; Differentiation

1. Introduction

The mammary gland is a complex organ that undergoes development and differentiation under the control of a number of hormone and growth factor ligands, their receptors, and some transcription factors. In rodents, the gland goes through four distinct stages of development: (1) rudimentary ductal structures appear in utero; (2) elongation and bifurcation of these primary ducts occur during puberty; (3) side-branching and alveolar buds increase with each estrous cycle; (4) lobuloalveolar structures capable of milk synthesis appear during pregnancy. The essential hormonal factors regulating the later two phases in mice have been established to be estrogen, glucorticoids and growth hormone during puberty, and estrogen, progesterone and placental lactogen and/or prolactin during pregnancy (Nandi, 1958; Neville and Daniel, 1987). The effect of these various hormones produces some development with each estrous cycle, and massive development at pregnancy. Following estrus or weaning this development never fully regresses resulting in ever increasing alveolar and ductal development with each episode (Vonderhaar, 1988). Circulating progesterone levels dramatically decrease at parturition, then nipple stimulation leads to milk secretion.

One of the most important recent advances for the study of mammalian genes has been the development of techniques to obtain defined mutations in mice. Thus, the use of mice deficient in cytokine, growth factor, receptor or transcription factor has permitted the identification of an ever-increasing list of molecules

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Table 1
Mammary phenotype in mice with targeted gene deletions

Gene	Mammary phenotype	Refs
Prolactin receptor	Lack of lobuloalveolar development	(Ormandy et al., 1997; Gallego et al., 2001)
Prolactin	Lack of lobuloalveolar development	(Horseman et al., 1997)
GHR receptor	Retarded ductal outgrowth	(Gallego et al., 2001)
EGF receptor	Very little ductal structures in fetal tissue	(Luetteke et al., 1999)
Progesterone receptor	Lack of terminal end bud formation, branching	(Lydon et al., 1995)
Estrogen receptor α	Lack of ductal growth and differentiation	(Korach et al., 1996)
Stat 5a	Failure of terminal differentiation	(Liu et al., 1997)
Stat 5b	Reduced development (some milk produced)	(Udy et al., 1997)
A-myb	Lack of lobuloalveolar development	(Toscani et al., 1997)
Cyclin D1	Lack of lobuloalveolar development	(Fantl et al., 1995)
SOCS-1	Accelerated development rescue of lactation in $PRLR^{-/-}$	(Lindeman et al., 2001)
Oxytocin	Unsuccessful milk ejection	(Young et al., 1996)

that affect mammary development and function. A non exhaustive list of mammary defects in knockout mice is summarized in Table 1. In addition, surgical techniques, such as transplantation of epithelial cells into cleared fat pads of recipient mice, have proven to be important in the identification of factors affecting mammary stromal or epithelial compartment.

2. Role of PRL and GH in reproduction

2.1. Reproductive status of PRL and PRLR knockout mice

It is widely accepted that lactogenic hormones play a major role in reproductive function, especially in rodents. Thus, it is not too surprising but comforting that female mice deficient in the PRL gene ($PRL^{-/-}$) are infertile. After mating with males of established fertility, no litters were produced following several matings. Each female mated repeatedly at irregular intervals, without entering a state of pseudopregnancy. Estrous cycles were irregular, and individual females failed to establish any consistent pattern of cycling. All these observations let to the conclusion that PRL is one of the hormones essential to female reproduction (Horseman et al., 1997).

PRL receptor $(PRLR)^{-/-}$ females also show an absence of pseudopregnancy and an arrest of egg development immediately after fertilization, with very few fertilized eggs reaching the stage of blastocysts (Ormandy et al., 1997). The outcome is a complete sterility. Uterine preparation for embryo implantation is dependent upon continued estrogen and progesterone secretion by the corpus luteum, which is supported by a functional pituitary during the first half of pregnancy in rodents (Astwood and Greep, 1938). PRL can directly stimulate ovarian progesterone secretion. Thus, whereas $PRLR^{-/-}$ females cannot implant blastocysts, the defect of the preimplantation egg development can be completely rescued by exogenous progesterone. However, although implantation occurs, full term pregnancy is not achieved (Binart et al., 2000).

Both PRL and PRLR genes are expressed in the uterus (Tanaka et al., 1996), suggesting that a paracrine/autocrine effect might be involved. Our observations indicate that preventing PRL action by disruption of the PRLR gene alters the maternal decidual transformation in response to the implanting blastocyst, demonstrating an essential role of PRL in reproduction. By using in situ hybridization and histochemistry techniques, PRLR specific hybridization signals were distributed over the decidual cells in early and term pregnancy (Reese et al., 2000).

Furthermore, the expression pattern of progesterone-dependent genes such as amphiregulin, COX-1, and Hoxa-10 was similar in wild-type and steroid-supplemented $PRLR^{-/-}$ mice. These results suggest that the correction of reproductive deficits by progesterone in $PRLR^{-/-}$ mice is accomplished by proper expression of progesterone-dependent genes that are essential in early pregnancy (Reese et al., 2000).

2.2. Reproductive status of GHR knockout mice

As has been previously reported (Zhou et al., 1997), litter size is markedly reduced in GH receptor (GHR) KO mice. We have recently evaluated reproductive function in female GHR KO animals. Reduction in litter size is attributed to an ovarian defect in these animals. IGF-I treatment is ineffective in rescuing this effect, suggesting that GH may act directly on the ovary (Bachelot et al., unpublished observations). Thus although reproduction is affected in GHR KO mice, pregnancy can proceed, rendering study of mammary development in these animals possible.

3. Role of PRL and GH in mammary development

Organogenesis of the mammary gland is completed when the ductal system has grown to the full extent of the fat pad and lobule buds have sprouted at regular intervals along the ducts. Up to midpregnancy, ductal elongation, branching, and the number of lobules increased. Under the influence of a combination of

systemic steroid and peptide hormones and local growth modulators, the lobuloalveolar epithelium undergoes extensive proliferation. At parturition, the lobuloalveolar epithelium is converted to a secretory phenotype and the full complement of milk proteins, lactose and lipogenic enzymes are synthesized. Involution of the lobuloalveolar system occurs at the end of the lactation in response to milk stasis, and decrease of systemic lactogens. Thus, GH, PRL and placental lactogens act during these different stages, inducing phosphorylation of Stat (Signal transducer and activator of transcription). Once activated, Stat5a and b proteins form homo- and hetero-dimers that translocate to the nucleus where they mediate the transcription of specific genes. Although the genes that are activated by these cytokines are largely unknown it is believed that they initiate programs of cell proliferation, differentiation and survival. In fact mice in which the Stat5a gene has been inactivated fail to develop functional glands during pregnancy.

Mammary development in $PRL^{-/-}$ and $PRLR^{-/-}$ mice is essentially blocked at the state of extended ductal outgrowths, which is understandable since most of the development of the mammary gland in mice occurs during pregnancy.

3.1. Mammary development and function in $PRLR^{+/-}$ mice

Interestingly, we originally reported that mice hemizygous for the $PRLR^{-/-}$ were incapable of producing enough milk for the newborns to survive, but in the second and subsequent pregnancies, or when the first pregnancy was delayed until the age of 20 weeks, the young survived (Ormandy et al., 1997). The impaired mammary development and alveolar differentiation seen during pregnancy in these hemizygous mice correspond to a reduction in receptor number and concomitant reduction of Stat5 phosphorylation levels, consequently the expression of milk protein genes is very reduced. Although a normal ductal network is formed during puberty, the fat pad is only sparsely filled with epithelium during pregnancy. The fact that these mice failed to lactate indicates that a threshold level of Stat5 phosphorylation is required for functional mammary gland differentiation. The degree of epithelial cell proliferation during pregnancy and the post partum period depends on a threshold of PRLR expression which is not achieved with just one functional allele, given that the level of PRLR is closely controlled in mammary gland (Ormandy and Sutherland, 1993). Hemizygous mice on a pure C57BL/6 background never lactate, even after multiple pregnancies. In contrast, in mice on a pure 129Sv background, mammary gland growth is insufficient to insure lactation at the first pregnancy but further estrous cycles or a single pregnancy lead to the development of a mammary gland capable of producing milk. This demonstrates either that continuous hormonal stimulus can overcome the block, or that compensatory mechanisms are established (Gallego et al., 2001).

3.2. Role of growth hormone in mammary gland development

Mammary transplantation experiments had shown that PRL-null epithelium forms normal ducts in wild-type hosts, but fails to develop lobuloalveolar structures during pregnancy demonstrating a cell autonomous defect of alveolar proliferation (Brisken et al., 1999). Although GH is not necessary for alveolar development, some lactogenic activity could be shown in cultured mammary epithelium from PRLR KO mice. Interestingly, ductal outgrowth in GHR-null mice was greatly retarded and side-branching was limited. After transplantation of GHR null epithelium into wild-type recipients ductal branching and alveolar differentiation were not different to wild-type animals. These experiments support the notion that GH signals through the stromal compartment.

3.3. Mammary development in $PRLR^{-/-}$ mice

Initial histological investigation of virgin glands of mature wild-type, $PRL^{-/-}$ or $PRLR^{-/-}$ animals indicated no dramatic differences, with ductal tissue present, confirming that PRL stimulus is not essential for this early state of development (Ormandy et al., 1997; Horseman et al., 1997). Because $PRLR^{-/-}$ females are sterile, the effect of the receptor mutation on mammary development during pregnancy must be analyzed in mice treated with progesterone or by transplanting $PRLR^{-/-}$ mammary epithelium into PRL wild-type mammary fat pads cleared of endogenous epithelial cells before puberty (Brisken et al., 1999). The combined results of multiple studies demonstrate that epithelial PRLR is not required for alveolar bud formation, but is absolutely essential for lobuloalveolar development (Gallego et al., 2001).

Taken overall, our findings demonstrate that GH and PRL activate Stat5 in separate compartments, which in turn reflects their specific role in ductal and alveolar development and differentiation (Gallego et al., 2001).

3.4. SOCS-1 and mammary gland

Recently, the SOCS (suppressor of cytokine signaling) gene family was identified as targets of the Jak/Stat pathway and was shown to encode proteins down-regulating this pathway at the level of activation. Although the involvement of individual SOCS proteins in PRLR signaling has been mainly studied using cell

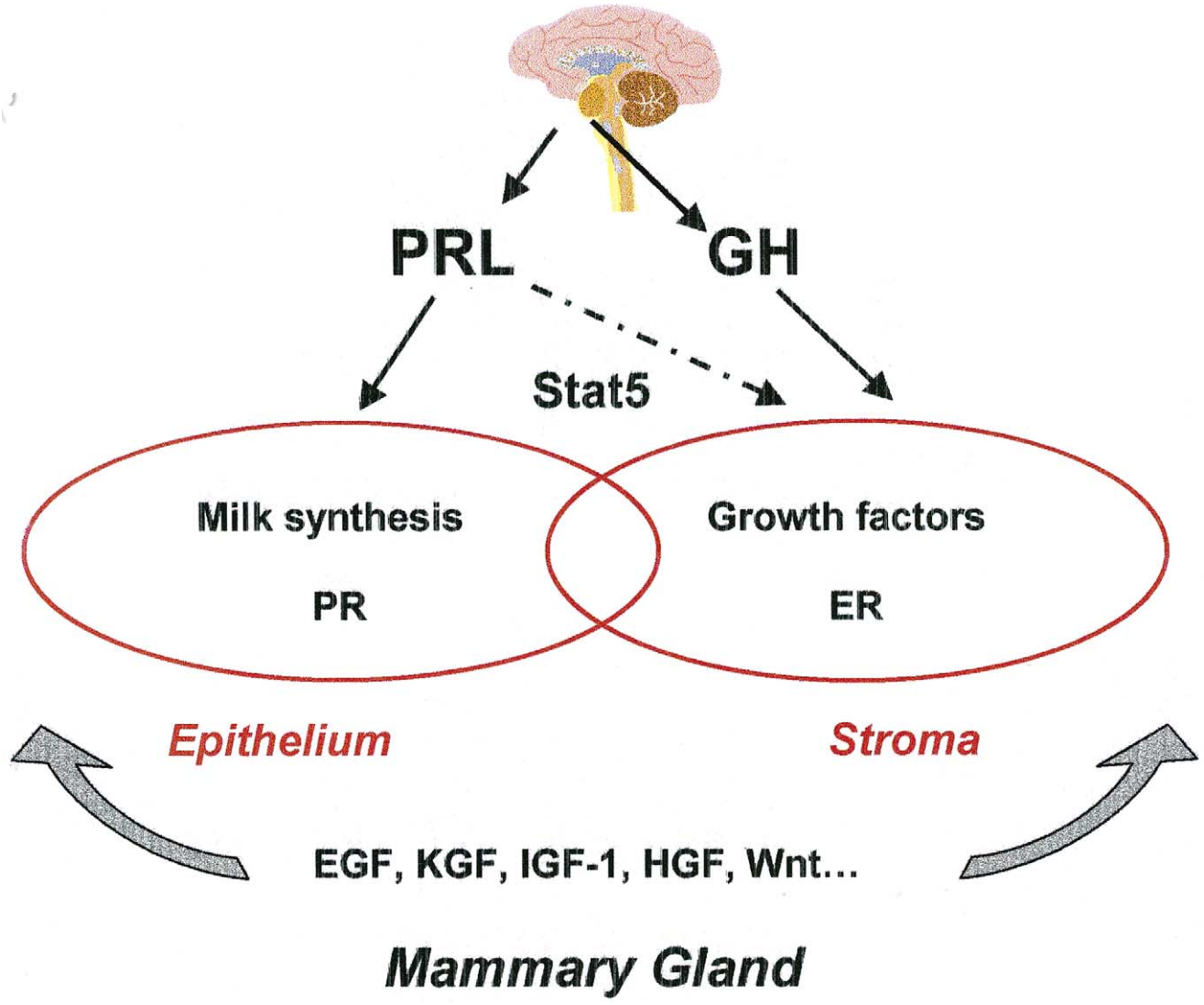

Fig. 1. Schematic representation of the PRL/GH actions on different compartments (epithelium and stroma) of mammary gland. The PRL/GH ligands meet their target cells (receptor activation, intracellular signaling via the nuclear factor Stat5). Then, specific effectors are activated either in epithelium or stroma (red circles) containing respectively progesterone receptor (PR) and estradiol receptor (ER). Moreover several growth factors indicated below the figure, are known to have specific and non redundant paracrine roles at different stages of mammary development.

transfection approaches (Tomic et al., 1999), the mechanisms by which endogenous SOCS regulate PRLR signaling in more physiological contexts are still to be elucidated (Barkai et al., 2000).

Among these suppressors of signaling, SOCS-1 has recently been proposed as a factor capable of preventing lactation prior to parturition. In fact, mice deficient for SOCS-1 which were rescued from neonatal death by concomitant deletion of the IFNγ gene, were shown to have accelerated mammary lobuloalveolar development. Interestingly, when a single allele of the SOCS-1 gene was deleted and these animals were crossed with $PRLR^{+/-}$ mice, the lactational defect normally seen in hemizygous mice was rescued (Lindeman et al., 2001). The functional pathways involved in SOCS-1 inhibition of the mammary gland remain to be identified.

4. Conclusions

In conclusion, the phenotypes of animals lacking functional genes encoding PRLR, PRL, GHR or Stat5 confirm the essential role of the lactogenic receptor and its signaling pathways and to a lesser extent the role of growth hormone in mammary gland development, whereas steroid receptors are also important but perhaps do not play as central a function as prolactin.

However, it is becoming clearer that a large number of genes play a role in mammary gland morphogenesis. In fact, the role of locally derived growth factors in the mediation of PRL-induced mammary gland development remains until unknown. Several growth factors are known to have specific and non redundant paracrine roles at different stages of mammary development.

These include epidermal growth factor (EGF), neuregulin, Wnt gene products, keratinocyte growth factor, hepatocyte growth factor and insulin-like growth factor-1 (IGF-1). The interplay between endocrine hormones and epithelial and stromal factors is essential for proper mammary development (Fig. 1).

In the future, it will be necessary to define the defects observed in branching morphogenesis and lobuloalveolar development at the molecular level. Thus, these model systems will be in the future crucial to identify the PRL regulated target genes important in the development of the mammary gland.

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